CYTOGENETIC STUDIES IN THE GENUS *ORNITHOGALUM* L.

IV. THE CYTOGENETICS OF INTER- AND INTRASPECIFIC CROSSES INVOLVING *O. THYRSOIDES* JACQ. AND *O. LACTEUM* JACQ.*

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ABSTRACT

Meiotic and fertility investigations were made of two inter- and intraspecific hybrids involving *O. thyrsoides* and *O. lacteum* in order to obtain cytotaxonomic data regarding the genetic relationship between the parental species.

No viable hybrids between the *O. lacteum* forma nov. from Sutherland ($2n = 10$) and other typical *O. lacteum* ($2n = 12$) biotypes have as yet been produced. However, viable, but sterile hybrids with *O. thyrsoides* from Bokbaai ($2n = 12$) have been obtained. It is doubtful whether the Sutherland form is in fact an *O. lacteum* biotype.

O. thyrsoides (Bokbaai) and *O. thyrsoides* (Sandveld) are two phenotypically very different biotypes, kept intact by mere geographical isolation.

INTRODUCTION

As pointed out by Stebbins, Dobzhansky, Lewis and others, (cf. Roos and Pienaar, 1966b) isolating mechanisms are fundamental in speciation. In studies concerning the relationship between taxa, the detection of barriers to gene exchange is of prime importance. The degree of gene exchange or its absence provides a measure of genetic relationship and enables a true classification.

Since Turesson's (1922) original studies in gene-ecology three biosystematic categories are being distinguished, viz. the cenospecies, ecospecies and ecotype, depending upon the absence or degree of gene exchange (cf. Clausen, 1951). A consideration of taxa in terms of these categories permits a more precise concept regarding their evolutionary relationship and taxanomic rank.

Since no major karyotypic differences could be detected between biotypes of *O. lacteum* and *O. thyrsoides* (Roos and Pienaar, 1966*a* and *b*) fertility studies and meiotic analyses were conducted upon their inter- and intraspecific hybrids in an effort to clarify their taxonomic relationship.

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MATERIALS AND METHODS

F_1 material from the following crosses and their parental species were fixed and treated as specified by Pienaar (1955): *O. thyrsoides* (Bokbaai) × *O. lacteum* forma nov. (Sutherland), *O. thyrsoides* (Bokbaai) × *O. thyrsiodes*) Sandveld).

RESULTS

From the large number of cross pollinations made between *O. lacteum* and *O. thyrsoides* biotypes the examples depicted in Table I give a good indication of the results obtained. Except for the hybrid no. 1269 mentioned below, no hybrids between the two species resulted from these reciprocal crosses.

Table I.
Reciprocal crosses between *O. lacteum* Jacq. and *O. thyrsoides* Jacq.

♂ ♀	*O. lacteum* Darling	*O. lacteum* forma nov. Sutherland	*O. thyrsoides* Bokbaai	*O. thyrsoides* Sandveld
O. lacteum Darling	+	—	—	—
O. lacteum forma nov. Sutherland	—	+	—	—
O. thyrsoides Bokbaai	—	×	+	×
O. thyrsoides Sandveld	—	—	×	+

× = viable hybrids.
— = no seeds set with viable embryos.
+ = pure bred plants.

Very few seeds were set in the cross between *O. thyrsoides* from Bokbaai ($2n = 12$; Fig. 1*a*) and *O. lacteum* forma nov. from Sutherland ($2n = 10$; Fig. 1*b*), producing hybrid no. 1269 ($2n = 11$; Fig. 1*c*). On the other hand, good seed set was obtained in the cross between *O. thyrsoides* from Bokbaai and *O. thyrsoides* from Sandveld ($2n = 12$; Fig. 1*d*), producing hybrid no. 1288.

Data of measurements conducted upon the pollen grains of the species and hybrids are summarized in Table II.

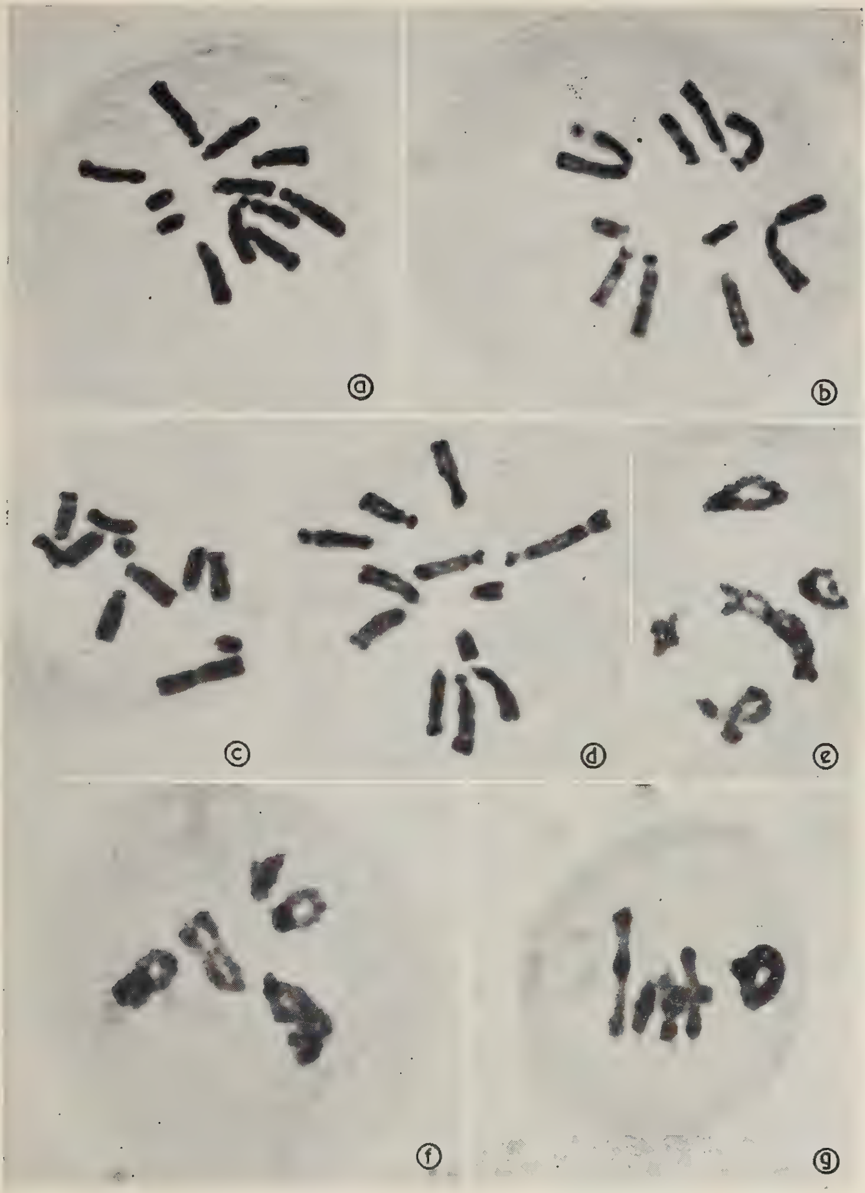

FIG. 1. (*a—d*) C-metaphase plates from root tip cells of *Ornithogalum* species and hybrids, × 2,000; (*a*) *O. thyrsoides* from Bokbaai; (*b*) *O. lacteum* forma nov. from Sutherland; (*c*) Hybrid no. 1269 = *O. thyrsoides* (Bokbaai) × *O. lacteum* forma nov. (Sutherland); (*d*) *O. thyrsoides* from Sandveld. (*e—g*) Meiotic stages in *O. lacteum* forma nov. from Sutherland, × 1500: (*e* and *f*) Diakinesis; (*g*) Metaphase I.

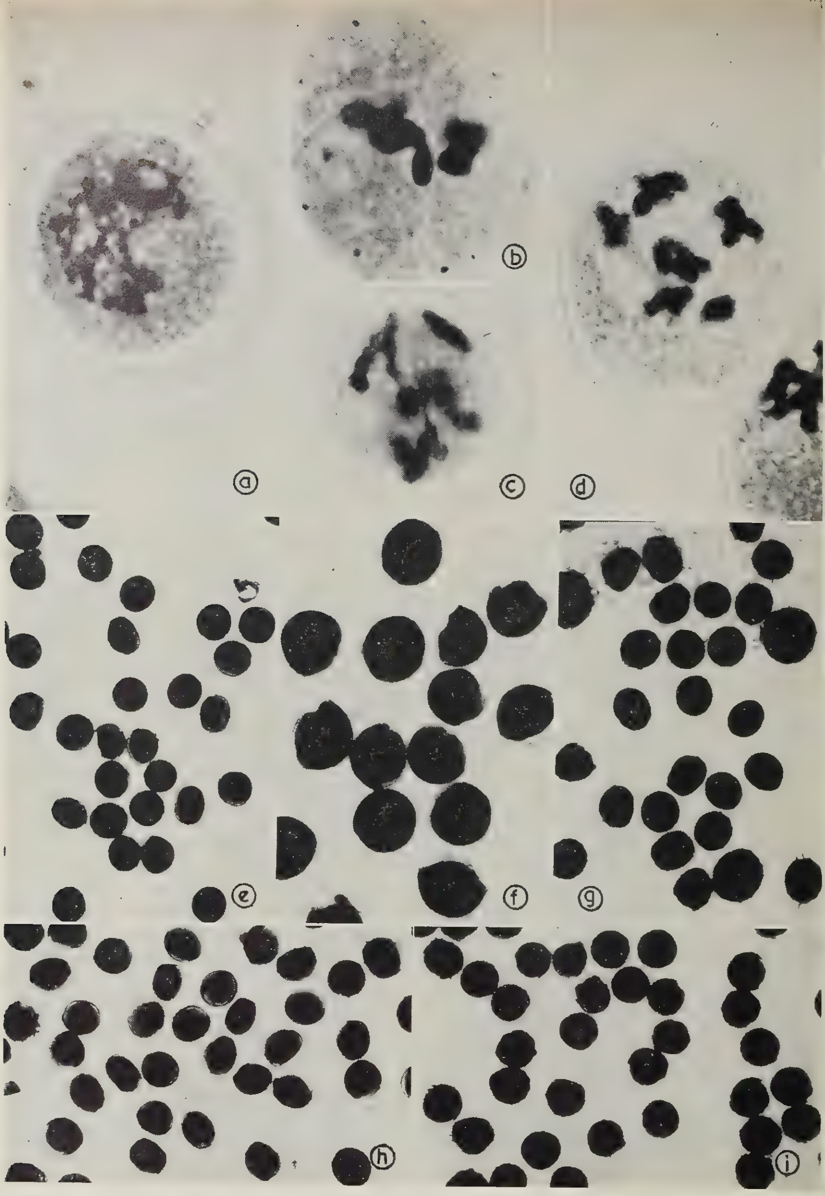

FIG. 2. (*a*—*c*) Meiotic stages in hybrid no. 1269, viz. *O. thyrsoides* (Bokbaai) × *O. lacteum* forma nov. Sutherland, × 1500; (*a*) Meiotically inhibited PMC from an advanced bud; (*b* and *c*) Metaphase I stages from a younger bud from a second plant; (*d*) Diakinesis in PMC from hybrid no. 1288, viz. *O. thyrsoides* (Bokbaai) × *O. thyrsoides* (Sandveld). (*e*—*i*) Glycerine-aceto-carmine stained pollen grains from various *Ornithogalum* species and hybrids, × 250; (*e*) *O. thyrsoides* from Bokbaai; (*f*) hybrid no. 1269, viz. *O. thyrsoides* (Bokbaai) × *O. lacteum* forma nov. (Sutherland); (*g*) *O. lacteum* forma nov. from Sutherland; (*h*) hybrid no. 1288, viz. *O. thyrsoides* (Bokbaai) × *O. thyrsoides* (Sandveld); (*i*) *O. thyrsoides* from Sandveld.

TABLE II.

NUMBER OF APPARENTLY VIABLE (CARMINE STAINED) AND INVIABLE POLLEN GRAINS, AND THE DIAMETER OF "VIABLE" POLLEN, MEASURED IN μ.

Plant	Number of pollen grains counted	Number of stained pollen grains	Percentage "viable" pollen grains	Mean diameter μ	Standard deviation
O. thyrsoides (Bokbaai)	533	512	96·1	55	± 3·5
O. lacteum forma nov. (Sutherland)	525	489*	93·1	57	± 5·2
Hybrid 1269	458	413	90·2	95	± 5·1
O. thyrsoides (Sandveld)	554	545	98·4	61	± 3·7
Hybrid 1288	705	653	92·6	61	± 3·6

* 7% of these pollen grains had a volume twice that of the haploid pollen grains and were considered to be diploid (cf. Fig. 2g).

Hybrid no. 1269 produced about 90·2% apparently viable pollen grains, all of which were exceptionally large (Fig. 2*f*). A chromosome count of their generative nuclei at late prophase revealed that the pollen grains were tetraploid. This rather irregular phenomenon was studied in detail by analysing a series of flower buds at successive stages of development. The series extended from pre-leptonema stages up to fully differentiated pollen grains.

Leptonema, zygonema and pachynema appeared normal. However, it was noted that very few microsporocytes passed the pachynema-diplonema stage. This stage was maintained in a few successive flowers, after which it appeared to revert back towards interphase (Fig. 2*a*). At about this stage the differentiation leading to the formation of the pollen grains started, and resulted in typically thick-walled pollen grains in which no meiotic reduction of chromosome number had taken place (Fig. 2*f*).

A few microsporocytes were noted in which meiosis did proceed to metaphase I and anaphase I (Fig. 2*b* and *c*). In these microsporocytes the chromosomes were clumped together to such an extent at diakinesis and metaphase I (probably due to the onset of degeneration), that no analysis of pairing and chiasma formation was possible. Some formed diads and very infrequently tetrads were produced, but all soon degenerated.

It appears therefore, that after a prophase reversal from the zygonema-pachynema stage, the majority of microsporocytes in this hybrid differentiate into unreduced pollen grains. The fact that the unreduced microsporocytes could continue development into pollen grains must be ascribed to their possession of a balanced tetraploid chromosome complement (cf. Roos and Pienaar, 1966*b*).

Data concerning the chromosome associations and chiasma frequencies at diakinesis of the *O. lacteum* forma nov. from Sutherland are summarized in Table III. From these data it is evident that in the *O. lacteum* forma with $2n = 10$, the total number of chiasmata per PMC (10·9) and mean number of chiasmata per bivalent (2·2) are similar to that found in *O. thyrsoides* ($2n = 12$) in which the respective chiasma frequencies are 12·7 and 2·1 (Table V). The large bivalent produced by the synapsed metacentric chromosomes had a chiasma frequency of 3·6, which was twice that of the mean number of chiasmata (1·8) occurring in each of the other four bivalents formed by the acrocentric chromosomes.

TABLE III.

CHROMOSOME ASSOCIATIONS AND CHIASMA FREQUENCIES AT DIAKINESIS OF *O. LACTEUM* FORMA NOV. FROM SUTHERLAND.

Chromosome associations		Number of cells scored	Number of chiasmata per bivalent*					Number of chiasmata per cell	Standard deviation	Mean number of chiasmata per chromosome pair
I	II		1	2	3	4	5			
	5	21	3·5	2·4	2·1	1·4	1·9	11·3	± 1·9	2·3
2	4	4	4·0	2·0	0·8	0·2	2·0	9·0	± 0·7	2·2
Total		25	3·6	2·3	1·9	1·2	1·9	10·9	± 0·8	2·2

* The individual bivalents could be distinguished on account of size differences and was numbered *1–5*; *1* being the bivalent formed by the two large metacentrics, and *5* the bivalent formed by the two smallest homologues.

Hybrid no. 1288 was an intraspecific cross between the *O. thyrsoides* biotype from Bokbaai as the female parent and the *O. thyrsoides* biotype from Sandveld as the male parent. Although the two biotypes are phenotypically very different, reciprocal crosses were readily made to give fertile hybrids (Table I). The Bokbaai biotype blooming early in October, has a 6″ peduncle suspending a raceme of large, wide opening, light cream coloured flowers having brown centres, while the Sandveld biotype, blooming late in November, is approximately 3′ tall and has smaller, white, cup-shaped flowers. These phenotypic differences were maintained even when both were subjected to the same environmental conditions.

In Table II it can be seen that hybrid no. 1288 formed 92·6% normal haploid pollen (Fig. 2*h*), which was very similar to that produced by the pure biotypes. A detailed study of the chromosome associations and chiasma frequencies at diakinesis and MI in this hybrid revealed a close similarity between the karyotypes of the two biotypes (Table IV and Fig. 2*d*).

Table IV.
Chromosome associations and chiasma frequencies at diakinesis of the hybrid *O. thyrsoides* (bokbaai) × *O. thyrsoides* (sandveld).

Chromosome associations				Number of cells scored	Number of chiasmata per cell	Standard deviation	Mean number of chiasmata per chromosome pair
I	II	III	IV				
	6			33	14·5	±1·05	2·4
2	5			17	11·9	±1·40	2·0
Total				50	13·6	±0·83	2·2

Although a few univalents were noted, mostly bivalent associations were formed. Compared to the chiasma frequencies in one of the parents (Table V), the mean of 13·6 chiasmata per cell in the hybrid is indicative of a close resemblance between the homologous chromosomes from the two parental biotypes.

Table V.
Chromosome associations and chiasma frequencies at diakinesis of *O. thyrsoides* jacq. (sandveld).

Chromosome Associations				Number of cells scored	Number of chiasmata per cell	Standard deviation	Mean number of chiasmata per chromosome pair
I	II	III	IV				
	6			16	12·9	±1·22	2·2
2	5			4	12·3	±0·47	2·1
Total				20	12·7	±1·19	2·1

The development of the pollen grains of hybrid no. 1288 were almost normal and reflected their balanced genomic constitution. The absence of detrimental interactions between the maternal cytoplasm and the reduced hybrid nucleus is further evidence of the genetic homology between the two biotypes.

DISCUSSION AND CONCLUSIONS

The hybrid between *O. thyrsoides* (Bokbaai $2n = 12$) and *O. lacteum* forma nov. (Sutherland $2n = 10$) must be regarded as exceptional in that this latter *O. lacteum* forma has as yet failed to produce hybrids with any of the other *O. lacteum* or *O. thyrsoides* ecotypes with which it was reciprocally crossed. No viable haploid pollen grains are formed by the hybrid. Potentially viable pollen grains appear to be tetraploid.

The pollen grains are tetraploid because no meiotic anaphase followed the chromosomal division during prophase in the PMC's; instead, the chromatids separate endomitotically causing the chromosome number in the nucleus to reduplicate. The fact that the unreduced microsporocytes can continue development into pollen grains must be ascribed to their possession of a balanced tetraploid chromosome complement (cf. Roos and Pienaar, 1966*b*).

A meiotic investigation of the diakinesis and MI stages in the *O. lacteum* forma from Sutherland (Table III, and Fig. 1*e—g*) did not reveal any abnormalities or polyploid PMC's. However, it is clear from fig. 2*g* that apart from the normal haploid pollen grains a fair number of diploid pollen grains are produced, in fact 7%. It is quite conceivable that the genetic factor which is responsible for this phenomenon is triggered into operation at an earlier stage in the genetically more unbalanced hybrid, causing the suppression of the post pachytene meiotic stages and inducing the PMC's to differentiate directly into the large tetraploid pollen grains. Although a few seeds were set by the hybrid after self-pollination and backcrossing, no progeny could be raised from them.

It was rather disappointing that meiotic pairing could not be followed in the hybrid as the paring between the large metacentric chromosome of the *O. lacteum* forma and the homologous chromosomes in the haploid set furnished by the *O. thyrsoides* parent, could have vindicated the hypothesis of Roos and Pienaar (1966*a*) regarding the origin of the metacentric chromosome in the karyotype of the *O. lacteum* forma. If their hypothesis, that this chromosome resulted from a centric union (due to a translocation in the centromere region) between two acrocentric chromosomes, is correct, then a trivalent consisting of the metacentric chromosome and two acrocentric chromosomes, with the three centromeres lying close together, should have been formed during meiosis. However, the fact that in the *O. lacteum* forma nov. the large bivalent formed by the metacentric homologues has a chiasma frequency twice that in each of the other bivalents (produced by synapsed acrocentric chromosomes) lends credence to the supposition that the metacentric chromosome resulted from a centric union between two acrocentric chromosomes and not from a pericentric inversion (cf. Table III).

In the light of the evidence given above it is considered unlikely that the *O. lacteum* forma nov. from Sutherland represents an *O. lacteum* biotype. Genetically it behaves like a separate species which can be cytologically indentified (Pienaar, 1963; Roos and Pienaar, 1966*a*).

The normal meiosis, gamete production and high fertility of the F_1 hybrid resulting from the cross between the Bokbaai and Sandveld biotypes of *O. thyrsoides* revealed the absence of an intrinsic barrier to gene exchange between them, and showed that these two morphologically distinct biotypes are kept intact merely by geographical isolation. Biosystematically they are two ecotypes of the same cenospecies.

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Addendum

In Part III of this series the symbol MI in tables III, IV, V and VI should be read to mean meiosis 1 (stage: diakinesis) and not metaphase I. On page 266 paragraph 1 read diakinesis instead of metaphase I.